3

Reprinted from the *Indian Medical Gazette*, Vol. XXXIII

(No. 1, Jan. 1898.)

FURTHER OBSERVATIONS ON THE TRANSFORMATION OF CRESCENTS.

BY

SURGN.-MAJOR RONALD ROSS, I.M.S.

Reprinted from the *Indian Medical Gazette*, Vol. XXXIII
(No. 1, Jan. 1898).

FURTHER OBSERVATIONS ON THE TRANSFORMATION OF CRESCENTS.

By Surgeon-Major Ronald Ross, I.M.S.*

In a former paper by me it was shown that the transformation of crescents into spheres and flagellate bodies occurs to a greater degree in the stomach of mosquitoes than in ordinary specimens. This I attributed at the time to some action of the insect's stomach on the parasite. Later, however, it became apparent that such action is not the real cause, as the following facts will show :—

(*a*) The transformation often occurs nearly as much in ordinary specimens.

(*b*) It occurs perhaps quite as much in the leech as is in the mosquito.

To explain more fully : in ordinary specimens, and even in different parts of the same specimen, it is seen that the percentage of crescents becoming transformed varies largely. Thus in one specimen only 10 per cent., say, will become spheres ; in another, 80 per cent.; in one part of a specimen we may find that scarcely any of the crescents have changed ; in another almost all. In the mosquito and leech, however, practically all the crescents develop into spheres. In other words, though the transformation is always most complete in the mosquito and leech, it may sometimes be nearly as complete in an ordinary specimen ; so that we can scarcely venture to claim for the insect's digestive organs that effect on the phenomena which I had at first believed it to possess. On the contrary, it is evident that the transformation depends upon some change in the condition of the blood after it is drawn from the capillaries—a change which, while it is found to an eminent degree in blood ingested by the leech and mosquito, is also found to a somewhat less degree in ordinary specimens. It remained then to ascertain what this change in condition really is.

---

* Read before the South Indian Branch, British Medical Association.

At the same time that I made the observation (appearing at first sight rather discouraging) that transformation occurs as much in the leech as in the mosquito, I also noted that, in ordinary specimens, it occurs to a great degree in the *presence* of *air-bubbles.* Thus in most ordinary specimens we may observe that those crescents which happen to be rather close to air-bubbles included in the blood under examination almost always become spheres ; while those crescents which lie at a distance from bubbles, or from the edge of the blood, generally remain crescents. Hence it seemed as if *exposure to air* encourages the transformation.

It was easy to ascertain whether this was the case. The drop of blood exuding from the finger-prick was left to stand there exposed to the air for various periods from 15 seconds to

4 or 5 minutes before being made into the specimen, and the various specimens so made were then compared. A most interesting and useful fact was elicited. The longer the blood was allowed to remain exposed to the air, the more crescents were transformed. Thus in blood which is exposed to the air for 15 seconds, only about 20 per cent. of the crescents become spheres, and those generally in proximity to air-bubbles. But in blood exposed for 2 or 3 minutes almost all the crescents, say, 90 per cent., proceed in development.

The same fact was next made even more strikingly manifest by the following experiments. A lump of vaseline was placed on the finger, and the skin was pricked through it, so that the escaping blood was protected on all sides from contact with air. The lump of vaseline with the droplet of blood included was then transferred to a slide and pressed out by a cover-glass. It was found that *none of the crescents became transformed.* Further, after an hour or so, the specimen was opened and the blood exposed to the air for a minute. *The transformation began immediately after exposure.* Again, a similar specimen was opened after 24 hours. Transformation no longer occurred : the crescents were dead.

These experiments have, of course, been repeated scores of times, or I would not make the statements so definitely. Lately, moreover, I have had several patients whose blood swarmed with crescents; so that it has been extremely easy to draw comparisons between specimens prepared in the different ways just indicated. I have also received the assistance of Dr. Srinivasa Row, B.Sc., &c., Mysore Medical Service, who will, I have no doubt, be able to confirm my statements.

We are now, however, struck by the thought that in the leech and mosquito, where the transformation is most complete, the blood is not exposed to the air at all! At first sight this appears to be absolutely contradictory of our former results. Let us, however, ask, how exposure to air can act on the crescents? It cannot be that they require the action of oxygen and nitrogen, because no such action is possible in the leech and mosquito. It must be that exposure to air acts by causing *evaporation of water from the blood.* If we hold a cold cover-glass over a drop of fresh blood, we shall find it covered with dew in a moment: evaporation from blood is very rapid.

But it will now be said evaporation of water from blood cannot possibly occur in the stomach of the leech and mosquito. No; but what is practically the same thing, *abstraction of water by digestion*, does occur most certainly. If we watch a feeding mosquito or leech, we shall observe that the first voids a little droplet of water every 10 seconds or so, and the latter sweats profusely; and if we examine blood which has been in their stomachs for even 10 minutes, we shall observe, by the greater huddling together of the corpuscles, that much of the water of the serum has been already drawn off by the digestive apparatus, evidently to allow room for more blood being ingested presently: hence, we may enunciate the following law with some confidence:—

Abstraction of a certain amount of water from the blood is a condition necessary to the transformation of crescents.

This may be confirmed by another experiment. The finger of the patient with a drop of blood hanging from it is quickly placed over warm steam, so as to check evaporation and even to add a little water to the blood, and is held in that position for one minute. Now, if the finger had not been held over steam, nearly all the crescents would have become transformed; as it is, only a few of them, namely, those generally which lie close to air-bubbles are changed. Here abstraction of water has been again checked, and so has the transformation.

Now what is the meaning of this? Why should abstraction of water have such a remarkable effect? It is merely another of those beautiful adaptations of animals to their external conditions which are so frequently found in nature.

Increased density, due to abstraction of water, is probably the only quality in which blood ingested by the mosquito differs at first from blood in the capillaries. When the blood is first taken into the mosquito it remains warm and still has a certain amount of motion; there is probably nothing mixed with it; and leucocytes and malarial parasites continue to live as before. But it does differ—and at once—in one respect; its density immediately begins to increase owing to the abstraction of water described above. As soon as the crescents feel this increased density, they commence their remarkable metamorphosis so as to be able to get it over before the rapidly-approaching viscosity, which takes place later, renders the serum too thick for the movement of their released flagellated spores. In other words the condition which determines the onset of the metamorphosis is just the condition which is found in the blood in the mosquito's stomach, and is used, so to speak, by the crescents as the signal that the time has now arrived for them to begin their metamorphosis. When we make an ordinary specimen there is always some evaporation from the serum between pricking the finger and applying the cover-glass; this evaporation causes a sudden small increase of density which leads a few deluded crescents into the belief that they have found their way into a mosquito, causing them to change at once. In such specimens, moreover, the air-bubbles abstract a further quantity of vapour which can be easily seen by the microscope as dew upon the cover-glass; hence, the crescents in the vicinity of such bubbles have still stronger grounds for their unfortunate impression. Lastly, in the vaseline experiment there is no evaporation or abstraction of water and consequently no crescents, or scarcely any, are transformed in it. Hence I think the explanation which I have given accords well with all the facts of the case, and may be accepted with some confidence.

But the above observations were only just completed when I saw a letter in the *Lancet* of the 24th October 1896 from Dr. Marshall, in which he says that the *addition* of a small quantity of water encourages transformation. At first sight this appeared to be directly contradictory of my observations, and it was therefore necessary to examine the question at once. My results are as follows:—

(1) The addition of so much water as makes red corpuscles swell up, converts crescents quickly into spheres.

(2) If too much water be added further development is checked, and the spheres are killed.

(3) If a small quantity be added, many of the spheres go on to exflagellation.

(4) If very little be added, so as just to compensate for loss of water by evaporation (as by exposure to steam), transformation is checked.

We have then to face the fact that, as Dr. Marshall says, the addition of a small quantity of water encourages transformation just as the *abstraction* of water does. The explanation of this appears to be very simple. It is, I think that any change of density starts the development of crescents, because it exerts a destructive, or at least an alterative, effect on the shell of the corpuscle containing the parasite. It is well known, for instance, that either increase or decrease of density, if sufficiently pronounced, causes extravasation of hæmoglobin. This alterative effect is evidently required by the crescents to assist them in liberating themselves from the shell of the corpuscle, so that as soon as they feel that the action is being exerted upon their envelope, they begin their transformation. But while condensation is the *natural* condition which produces this effect, being found in the stomach of the mosquito, attenuation by means of water has the same result *accidentally*, so to speak, because it happens to produce the same effect on the shell of the corpuscle. I dare say many other re-agents may be found hereafter, which will similarly control transformation one way or another.

A good parallel to the above is found in the fact, discovered by Manson, that extravasation of hæmoglobin is required to enable the filaria embryo to cast its sheath. This extravasation is produced naturally in the stomach of the mosquito, I think, by abstraction of water. But we may produce it artificially, by cold, and, I daresay, by other means. While abstraction of water is, therefore, the natural means of enabling the embryos to cast their sheath, cold, &c., may be considered as artificial substitutes. It would be interesting to make a series of experiments with a view to comparing the process of casting the sheath in the filaria and the malaria parasite respectively. That the latter casts a sheath I hope to show in my next paper, in which I propose to detail the exact stages of transformation of the crescent.

In concluding this paper, however, I wish to point out that the fact that transformation will not occur at all unless the density of the blood is changed deals a final blow to the theory of Antolisei, Bignami, Bastianelli and others, that exflagellation is a process of degeneration due to death. In the vaseline experiment, for example, all the parasites die after 24 hours, but none of them become flagellate ! The process, therefore, cannot be one of death ; it must be one of *life*, and being such, must have some significance in the life-history of the parasite.

The same fact, too, strongly encourages the view that transformation is meant to occur in the stomach of the mosquito, because transformation depends on a change of density of the blood, and a change of density is just the one condition by which fresh mosquito-injected blood differs from blood in the capillaries. As already mentioned, this looks like a case of adaptation of habits and structure to external conditions ; and I cannot help thinking that the fact is one of the most promising which have yet been adduced in favour of Manson's theory.